Ileal and total tract digestibility and nitrogen utilisation in blue fox (Vulpes lagopus) fed low-protein diets supplemented with DL-methionine and L-histidine

Ylinen, Vappu

2020-05-03

Ylinen , V , Pylkko , P , Peura , J & Valaja , J 2020 , ' Ileal and total tract digestibility and nitrogen utilisation in blue fox (Vulpes lagopus) fed low-protein diets supplemented with DL-methionine and L-histidine ' , Archives of Animal Nutrition , vol. 74 , no. 3 , pp. 237-255 . https://doi.org/10.1080/1

http://hdl.handle.net/10138/324779
https://doi.org/10.1080/1745039X.2020.1716655

acceptedVersion

Ileal and total-tract digestibility and nitrogen utilisation in blue foxes (*Vulpes lagopus*) fed low-protein diets supplemented with DL-methionine and L-histidine

Vappu Ylinen[a,*], Päivi Pylkkö[b], Jussi Peura[c], Jarmo Valaja[a]

[a] Department of Agricultural Sciences, University of Helsinki, Finland

[b] Kannus Research Farm Luova Oy, Kannus, Finland

[c] Finnish Fur Breeders' Association, Vantaa, Finland

* Corresponding author, E-mail address: vappu.ylinen@helsinki.fi

Abstract

A lower dietary protein supply for adult blue foxes has been suggested. To formulate a low-protein diet with sufficient amounts of amino acids (AAs), AA digestibility and the AA requirement of the animals are crucial information. Therefore, a digestibility and nitrogen (N) balance trial was conducted with 20 blue foxes to determine the macronutrient and AA digestibility and N utilisation in low-protein diets supplemented with DL-methionine (Met) and L-histidine (His). In addition, plasma urea and plasma AAs were measured. The diets were designated as P24 (control), P20, P20M, P16M and P16MH and contained digestible crude protein (DCP) amounting to 24%, 20% or 16% of metabolizable energy (ME). The 20% protein level was fed with or without Met and the 16% protein level was fed with Met and with or without His. The apparent total-tract digestibility (ATTD) of crude protein linearly decreased with decreasing dietary protein level. The ATTD of dry matter, organic matter and crude carbohydrates increased when wheat starch was added as a replacement for protein. The apparent ileal digestibility (AID) and ATTD methods were compared to determine the AA digestibility. The decreasing dietary protein supply decreased the ATTD of most of the AAs: threonine, tryptophan (Trp), valine, alanine (Ala), aspartic acid (Asp), glutamic acid, glycine (Gly), proline (Pro), serine (Ser) and total AAs. The AID of the AAs was constant between diets. Diverging AAs showed higher or lower digestibility when determined in the AID or ATTD methods. Isoleucine, lysine, Met, Ala and tyrosine showed higher levels of AID. Arginine, His, cysteine (Cys), Trp, Asp, Gly, Pro and Ser showed higher levels of ATTD, which may reflect the net loss of these AAs in the large intestine. Met and His supplementation improved the ATTD and AID of the AAs in question, respectively, but did not affect the other variables examined. N retention did not differ between diets and renal N excretion decreased with decreasing protein level; thus N utilisation improved. We concluded that the protein supply and AA composition in low-protein diets with supplemented Met were adequate for adult blue foxes, since the lower protein

supply improved N utilisation and did not affect N retention. However, His supplementation failed to reach the designed level and therefore showed no clear results.

key words: amino acid; digestibility; blue fox; carnivore; nitrogen utilisation

1. Introduction

Reduced dietary protein for more efficient nitrogen (N) utilisation, lower ammonia emissions and the feasibility of using low-cost or alternative feed ingredients are targets of both ecological and economical animal production. Feed formulation based on the animal's amino-acid (AA) requirements and digestible AAs is one of the key factors contributing to these targets. Farmed blue fox (*Vulpes lagopus*), as a carnivore species used for fur production, is traditionally fed with high protein diets. As high quality fur is the main product of this fur production, AA content and especially sulphur-containing AAs contributing markedly to fur growth, is one of the main interests when developing feeds for fur animal species. The current recommendations for protein feeding for blue foxes are based on the proportion of digestible crude protein (DCP) of metabolizable energy (ME). The protein evaluation system takes into account the calculated AA composition of the feed, but the protein and AA requirement of blue foxes at the late growing-furring period, when the winter-fur in priming are not completely know. However, taking account the AA requirement of the animal at different life stages might bring both economic and environmental benefits and allow the use of alternative dietary protein sources, as has been shown in other species such as poultry and swine (Williams 1995, Perttilä et al. 2002, Lemme et al. 2004, Aarnink & Verstegen 2007).

The current protein recommendations for fast-growing blue foxes in the late growing-furring period (from early September until pelting) is in Finland min. 22 % of metabolizable ME (Profur 2019). Previous recommendation has been established by Lassén et al. (2012), when it was 24% of ME. However, lower protein levels, even as low as 15 % of ME, has been suggested (Dahlman 2003, Ylinen et al. 2018). Particularly the lower protein levels could be used in the latter part of the late growing-furring period. However, when the dietary protein supply is limited, the AA composition should be balanced to meet the animal's AA requirements, since no excessive protein or AAs are present. Research is needed to increase our understanding of the limiting AAs and AA digestibility

in blue foxes. AA digestibility in blue foxes varies, depending on the protein sources (Skrede et al. 1980). Protein sources with low digestibility vary more widely in digestibility between individual AAs than highly digestible protein sources (Skrede et al. 1980). Use of low-digestible protein sources, such as meat-and-bone meal or alternative feed ingredients to replace more traditional ingredients, such as fish, is growing in the fur feed industry. To ensure sufficient AA supply, it is crucial to determine the AA digestibility coefficients and formulate the feed based on these data.

In the present study, AA digestibility was determined using both the apparent total-tract digestibility (ATTD) and apparent ileal digestibility (AID) methods. Reliable evidence suggests that in other monogastric species the AID rather than the ATTD is considered the preferred method of determining AA digestibility, because the microbial fermentation in the large intestine affects both the quantity and composition of faecal protein and AAs (Mosenthin et al. 2000, Sauer et al. 2000). Most of the data concerning the differences between AID and ATTD have been carried out in swine, but studies have also been conducted in minks, dogs and blue foxes (Szymeczko 2001, Hendriks & Sritharan 2002, Vhile et al. 2005, Tjernsbekk et al. 2014). These predominantly carnivore species have short colons with limited microbial fermentation capacity, and it was therefore expected that the hindgut effect would not be significant. In dogs, the AID method has been considered more accurate (Hendriks & Sritharan 2002, Hendriks et al. 2012, Tjernsbekk et al. 2014), while in blue foxes, the ATTD method has been primarily used (Dahlman 2003) and N disappearance from the large intestine is minimal (Hendriks et al. 2012). However, in blue foxes, the AID method has sometimes resulted in lower apparent AA digestibility, which may reflect the net loss of AA in the large intestine and overestimation of AA digestibility carried out in the ATTD method (Szymeczko 2001, Vhile et al. 2005). We determined AA digestibility using ATTD and AID to compare results, expecting to see higher digestibility values in ATTD than AID. Our general aim was to increase knowledge of the true digestible AA requirement of blue foxes. As ATTD is

less invasive method than AID and possible to conduct with intact animals, it would be beneficial to be able to calculate the true AA digestibility from results gained using ATTD method. Therefore, our objective was to compare AID and ATTD AA digestibility in blue foxes to determine differences for single AAs that can be applied for estimating AID values from ATTD values.

In addition to the digestibility trial, an N balance trial was conducted to detect the effect of the experimental diets on N retention. Our hypothesis was that lowering the protein supply improves N utilisation and can thus decrease N excretion, as has been shown in various monogastric species, including blue foxes (Dahlman et al. 2002a, Otto et al. 2003, Belloir et al. 2017). Our aim was to used N balance to show, that can the dietary protein levels be reduced without affecting N retention and if dietary supplementation of Met and His to low protein diets will increase N retention.

We determined the nutrient and AA digestibility and N utilisation in adult blue foxes fed low-protein diets with or without DL-methionine (Met) and L-histidine (His) supplementation. The study was conducted in two separate terms in late growing-furring period to define more closely the protein and AA requirement of the blue fox, and thus elaborate on the results of Dahlman et al. (2002a) which covered the entire growing-furring period. Met is the first limiting AA in fur animals and, accordingly, was selected to be supplemented in the low-protein diets in this study. Based on the Dahlmans (2002a) study, we hypothesised that the protein level 16% of ME needs to be supplemented with Met but wanted to see the effect of supplementation at protein level 20% of ME. His was selected as the second experimental supplemental AA as it was the third limiting AA in the study of ideal protein for blue foxes (Dahlman et al. 2004). In the present study, the AID and ATTD methods were compared to determine differences for single AAs that can be applied for estimating AID values from ATTD values. The study aims for improved feed formulation, based on digestible

AAs and the animal's AA requirements, which could result in economic and environmental benefits for the production sector.

2. Materials and methods

2.1 Experimental diets and feeding

The digestibility and N balance trial was conducted at the Kannus Research Farm Luova Oy, Kannus, Finland. Three dietary protein levels were studied as a part of the performance trial carried out with the feeds in question (Ylinen et al. 2018). Two lowest protein levels were studied with or without Met and His supplementation. The control diet and four experimental diets were designated as P24, P20, P20M, P16M and P16MH. The control diet (P24) contained DCP amounting to 24% of ME. The experimental diets contained DCP amounting to 20% of ME with and without Met supplement (P20 and P20M) and 16% of ME with Met and with Met and His supplements (P16M and P16MH). The Met and His supplementation was calculated to bring the AA in question to the same level as in group P24. Celite® (SiO_2), was added at 0.6% as a marker. The feed ingredients are shown in Table 1. The daily feeding ratio was on a dry matter (DM) basis 298 g DM, fed once daily at noon. Drinking water was freely available.

2.2 Animals and experimental design

The trial was conducted in autumn 2016 with 20 blue foxes males of 23 weeks of age..The average body weight was (± standard error of the means SEM) 15.5 (±0.2) kg. The animals were in the hall environment housed individually in wire-mesh metabolism cages for controlled feeding and quantitative collection of faeces and urine. The animals were randomly distributed on five groups giving four replicates for each diet. Before the experiment, the animals were fed a basic farm diet. The trial period comprised four days of adjustment and three days of total collection of faeces and

urine. The daily feed consumption was recorded and potential residual feeds collected, weighed and analysed. The chemical composition of the feeds was analysed in pooled samples collected daily throughout the collecting period. Total faeces were collected daily prior to daily feeding. Total urine was collected into containers added four ml of 10 N sulphuric acid to prevent ammonia evaporation. Samples of the feed, faeces and urine were kept frozen (–20°C) pending analysis. Digestibility of DM, organic matter (OM), crude protein (CP), crude fat (CF), crude carbohydrates (CCH) and ash was determined using acid-insoluble ash (AIA) as an inert marker. To determine AA digestibility, both the ATTD and AID methods were used, using AIA as a marker. To determine AID, the animals were euthanized after the total collection period. Engine euthanasia was performed with filtered exhaust gases (combination of CO, CO2, HC, O2); CO concentration in the euthanasia chamber being 4%. Euthanasia was performed individually, 3.5 h after the last feeding. After euthanasia, the digestive tract was exposed and the ileum dissected and divided into three equal segments. The digesta of the terminal third of the ileum were gently squeezed out of the gut and immediately frozen at –20°C. In addition, urine urea, urine N, plasma urea and plasma AAs were recorded. For blood samples, the venous blood was collected once on the last total collection day. Blood was sampled from the cephalic vein 24 h after last feeding.

2.3 Calculations

The apparent digestibility of the macronutrients and AA was calculated as follows:

ATTD or AID (%) = (1 – (AIA in nutrient/AIA in faeces or ileal digesta) * (nutrient in faeces or ileal digesta/nutrient in feed)) * 100.

The N balance parameters were calculated as follows:

N absorbed = N intake – Faecal N,

N retained = N intake – (Faecal N + Urinary N),

In addition, the N retained was calculated as the percentage of intake and absorption and as grams per kilogram live weight.

The ME (MJ/kg) content of the feeds was calculated, using the chemical composition and digestibility coefficients carried out with the feed in question and ME (MJ/kg) values: protein (N x 6.25) 18.8; fat 39.8; carbohydrates 17.6 (Lassén et al. 2012). The energy distribution was calculated according to Lassén et al. (2012).

2.4 Chemical analysis

The samples were pooled within diet (feeds) or animal (faeces and urine) or analysed as single samples (ileal digesta, blood). The faecal samples were homogenized, using a hand-held electric mixer. The analyses were conducted in the Laboratory of Agricultural Sciences, University of Helsinki, Finland, except the CF, which was analysed in the Laboratory of Fin Furlab Oy/Ab , Vaasa, Finland and plasma urea, which was analysed in the Laboratory of Veterinary Medicine, University of Helsinki. The chemical composition of the feed and faeces was analysed by standard methods according to the AOAC International (1995). Dry matter was determined by oven drying at 103°C for 24 h and crude ash was determined at 600°C for 24 h. The feed and faecal samples were dried using lyophilization to prevent protein breakdown. The hair was sifted, and the dry samples were ground in a porcelain mortar in preparation for analysis. The CP was determined by the Kjeldahl method (AOAC International, 1995) with a Tecator Auto Digestion unit and a Kjeltec Auto 2300 Analyser (Foss A/S, Hillerød, Denmark). The CF was determined by solvent extraction according to the Weibull-Stoldt technique (BÜCHI Hydrolysis Unit B-411 and BÜCHI Extraction Unit B-811; BÜCHI Labortechnik AG, Flawil, Switzerland). The CCH were calculated as the difference obtained by subtracting the ash, CP and CF from the DM. Urinary urea was determined with a HUMAN Liquicolour Complete Test Kit and spectrophotometer (Shimadzu ultraviolet (UV)

191 mini 1240; Shimadzu Corp., Kyoto, Japan). AA were determined by ultraperformance liquid
192 chromatography (UPLC) method as described in Puhakka et al. (2016) was used. Prior to AA
193 analysis, the samples were hydrolysed according to Directive 98/64/EC (European Commission,
194 1998). Venous blood collected in tubes containing ethylenediaminetetraacetic acid (EDTA) was
195 centrifuged at 1000–-1300 G for 10 min. Following centrifugation, the plasma was removed and
196 frozen at –20 °C pending analysis. For AA analysis, the plasma samples were precipitated with
197 10% sulphosalicylic acid and further analysed by UPLC, as described above for the hydrolysed feed
198 samples. Plasma urea was analysed using a Konelab 60i analyser (Thermo Fisher Scientific,
199 Waltham, MA, USA).
200
201 **2.5 Statistical analysis**
202 Statistical analysis of the data was performed with the general linear model (GLM) procedure of
203 SAS 9.4 (SAS Institute Inc., Cary, NC, USA). The data were tested for normal distribution, using
204 the Shapiro-Wilk test and the homogeneity of variance with Levene's test. The model used in
205 analysis was:
206 $$Y_{ij} = \mu + d_i + \varepsilon_{ij},$$
207 where Y_{ij} = the observation, μ = the general mean , d_i = the effect of diet (I = 1, …, 5) and ε_{ij} = a
208 random effect. The diet effect was tested, using four orthogonal contrasts: C1 tested the effect of the
209 control diet (P24) against the experimental diets and C2 the effect of the protein level between
210 groups P20 and P16. C3 tested the effect of Met supplementation between groups P20 and P20M,
211 and C4 the effect of His supplementation between groups P16M and P16MH. In the analysis of the
212 method effect, the average ATTD and AID across diets was used. The effect of the method and
213 interaction between the diet and method were tested, using the GLM-procedure of SAS 9.4.
214
215 **3. Results**

All animals were healthy, with normal faecal consistency throughout the experiment. The feeds were eagerly consumed, and no feed residuals were detected. One animal in group P20M and one in group P16MH showed extremely low protein digestibility values (< 2 SD), and their results were excluded from the macronutrient digestibility and N balance analysis as outliers. However, the digestibility values of individual AAs were within the normal range in these two animals, and all AA results were included in the analysis.

3.1 Feeds

The experimental diets followed the designed levels, with some differences (Table 2). The total CP content (g/kg DM) of the diets followed the levels designed, except in group P16MH, where the total CP was lower than that designed. The DCP as a percentage of ME was lower than that designed in all groups. On average, the DCP content was 21%, 17% and 15% of the ME in diets P24, P20 and P16, respectively. Energy supply was 5.4 – 5.8 MJ ME per day per animal. Since the energy distribution of the macronutrients was calculated, using the digestibility values obtained from this study, the low protein digestibility may have affected the low DCP content as a percentage of the ME.

The CF content (g/kg DM) was lowest in groups P24 and P16MH. The AA content of the diets (g/kg DM) is presented in Table 3. The low CP content reflected the low AA content of diet P16MH (total and individual AAs). The Met content in groups P16M and P16MH exceeded that of group P24. As planned, the lowest Met content was in the diet not supplemented with Met (P20). In contrast, the His supplemented in group P16MH did not reach the level of the group P24, but was higher than in the nonsupplemented groups.

3.2 Apparent macronutrient and amino-acid digestibility

241 Foxes fed the P24 showed higher ATTD of CP than with all other diets ($p = 0.01$, Table 4). In
242 group P16MH, protein digestibility was especially low. The ATTD of CCH, DM and OM was
243 higher in groups P16 than in groups P20 ($p < 0.0001$, $p = 0.0003$ and $p = 0.005$, respectively). The
244 ATTD of CF was high (over 94%) in all groups, but lower in group P24 ($p = 0.01$) than in all other
245 groups. Met and His supplementation did not affect the macronutrient digestibility coefficients.
246
247 The AID of Met increased by Met supplementation ($p = 0.0007$, Table 5) and His supplementation
248 positively affected the AID of His ($p = 0.04$). The AID of Met was lower in group P24 than in the
249 Met-supplemented groups ($p = 0.04$) and higher in group P16 than P20 ($p = 0.003$). All other AAs
250 and total AAs showed no differences in AID among diets. The ATTD of the AAs decreased with
251 decreasing protein level for the following AAs and total AAs: threonine (Thr), tryptophan (Trp),
252 valine (Val), alanine (Ala), aspartic acid (Asp), glutamic acid (Glu), glycine (Gly), proline (Pro) and
253 serine (Ser) ($p \leq 0.05$, Table 6). The ATTD of Met increased with Met supplementation ($p = 0.001$).
254 His supplementation did not affect the ATTD of His between P16M and P16MH, but the ATTDs of
255 Met and His were higher in group P16 than in group P20 ($p = 0.001$).
256
257 **3.3 Apparent total tract and apparent ileal digestibility amino acid values**
258 The average ATTD and AID of AAs throughout the diets are shown in Table 7. The diverging AAs
259 showed higher or lower digestibility when determined in the AID or ATTD methods. For arginine
260 (Arg), cysteine (Cys), His, Trp, Asp, Gly, Pro and Ser, the ATTD method resulted in higher
261 digestibility coefficients ($p \leq 0.05$). For isoleucine (Ile), lysine (Lys), Met, Ala and tyrosine (Tyr),
262 the AID method resulted in higher digestibility coefficients ($p \leq 0.05$). No diet method interaction
263 was found.
264
265 **3.4 Nitrogen utilisation and plasma amino acids**

N intake, urinary N excretion, urea N (g/d) and absorbed N decreased with decreasing dietary protein level ($p < 0.0001$, Table 8). Urea N as a percentage of total urinary N or daily retention of N (as grams per day, grams per live weight, percentage of intake or percentage of absorption) did not differ between groups. No differences between diets were found in plasma urea.

The content of the following AAs in plasma decreased with decreasing protein level ($p \leq 0.05$): Ile, leucine (Leu), phenylalanine (Phe), Thr, Trp, Val, Asp and Tyr (Table 9). The content of plasma Met and His was constant among the diets.

4. Discussion

In general, composition of the diet and related factors, such as feed intake, affect the digestibility of main nutrients and AAs (McDonald et al. 2011). In developing feeds or optimizing diets, changes in the diet and therefore in digestibility may markedly affect nutrient availability. In blue foxes, changes in protein composition are especially important, since the quality of the pelt is dependent on the protein level and AA composition of the diet (Dahlman et al. 2002a, 2002b). At the same time, economic and environmental issues compel the reduction in protein use in fur animal feeds.

In the present study, CP ATTD linearly decreased with decreasing CP of the diets. In a previous digestibility study in adult blue foxes fed low-protein diets, the protein digestibility has correspondingly decreased with decreasing dietary protein levels, from 83% to 78% and from 86% to 79% (Dahlman et al. 2002a). The low-protein levels used in that study were 22.5 and 15% of ME and control protein level 30% of ME (Dahlman et al. 2002a). Opposite to our study, the supplemented Met improved the digestibility of the DM, ether extract and CCH in the low-protein diets in the study of Dahlman et al. (2002a).

In both studies, digestibility was determined, as apparent, and thus endogenous and microbial protein was not taken into account. Studies in swine have shown that apparent digestibility values

291 are dependent on the dietary protein level, since the relative proportion of basal endogenous and
292 microbial protein in the digestive tract is higher when the dietary protein supply is lower
293 (Mosenthin et al. 2000). The effect is quadratic and greater when the protein supply is minimal (Fan
294 & Sauer 1997). Similarly, it have been obtained in blue foxes and minks that lower dietary protein
295 decreases apparent protein digestibility, and probably the effect of basal endogenous and microbial
296 protein has diluting effect on digestibility values (Skrede et al. 1980, Szymeczko & Skrede 1990,
297 Dahlman et al. 2002a). In our study, the effect of endogenous protein may have been pronounced in
298 the lowest protein group, where the protein supply was lower than that designed and the
299 digestibility value was low.
300
301 Higher ATTD of DM, OM and CCH was observed in group P16 than in P20. In P16, the proportion
302 of cooked wheat starch, the main replacement for the decreased protein, was higher than in P20.
303 Cooked wheat starch has a high digestibility of carbohydrates (87%) (LUKE 2006). The higher
304 proportion of starch likely increased the ATTD of CCH and further influenced the ATTD of DM
305 and OM. In the previous digestibility study in blue foxes (Dahlman et al. 2002a), the ATTD of
306 CCH, DM and CF decreased with decreasing dietary protein. In their study, the ingredients
307 replacing the decreasing protein were wheat bran and cooked wheat starch (in 1:2 ratio,
308 respectively). Fibre is known to decrease the digestibility of almost all nutrients and energy by
309 increasing passage rate and withholding the nutrient absorption by adsorption of nutrients into fibre
310 (Sauer et al. 1991, Wenk 2001). In addition, especially fermentable fibres tend to increase microbial
311 fermentation in the large intestine and thus microbial protein in the faeces, reducing the ATTD
312 values (Sauer et al. 1991, Silvio et al. 2000). In our study, the crude fibre content was not analysed,
313 but since the addition of wheat bran was low and the proportion equal between diets, we assumed
314 that it did not markedly interfere with the digestibility values between diets.
315

316 The ATTD of AAs showed that the digestibility of nonessential AAs (Ala, Asp, Glu, Gly, Pro and
317 Ser), some essential AAs (Thr, Trp and Val) and total AAs decreased. Met and His supplementation
318 increased the ATTD of the AAs in question, respectively. The results accorded with previous
319 studies, in which the supplemented AAs were thoroughly and rapidly digested (Wang & Fuller
320 1989, Dahlman et al. 2002a). In the study of Dahlman et al. (2002a), decreasing AA digestibility
321 along with decreasing protein levels were found for all AAs except Cys. In addition, Met and Lys
322 supplementation increased Met and Lys digestibility, respectively. In both studies, AA digestibility
323 was determined as apparent in lieu of true digestibility. As in macronutrient digestibility, we
324 presumed that the faecal endogenous protein affected the decreasing ATTD of the AAs with
325 decreasing protein level in the ATTD method. However, the diluting effect of endogenous protein
326 to digestibility values was not seen in the AID values, even though the ileal digesta contains
327 endogenous proteins such as bile, digestive enzymes and cells sloughed off in the mucus. As shown
328 in the study of Szymeczko & Skrede (1990) in mink, endogenous secretions already affect the AA
329 composition of the digesta in the first section of the small intestine.
330
331 The AID of the AAs did not differ between diets, with the exception of Met and His, since the
332 supplementation of the AAs increased the AID of the AA in question. In addition, Gugołek et al.
333 (2017) reported improved protein digestibility and N retention with abundant Met supplementation.
334 In our study, Met supplementation was slightly lower than in the study of Dahlman et al. (2002a)
335 and considerably lower than in Gugołek et al. (2017). However, in Gugolek et al. (2017) the aim
336 was to study the effects of extremely high Met supplementation and in our study we pursued the
337 lowest possible Met supplementation. In our study, the digestible Met was in low protein diets 0.32
338 g/MJ ME. Dahlman et al. (2003) concluded that adequate digestible Met should be 0.40 g/MJ ME
339 in blue foxes in late growing-furring season. In the present study, His supplementation was,

unfortunately, inadequate, since the His content of the supplemented diet did not reach the level of the control diet (P24), making it impossible for us to conclude on the effect of His supplementation.

In our study, the variation in AA digestibility results was greater in the AID than in the ATTD methods. Muir et al. (1996) and Murray et al. (1997) obtained correspondingly greater variation, using the AID method in ileal-cannulated (simple T-type cannulas) dogs. Muir et al. (1996) concluded that a possible source of error could have been the marker (chromic oxide) used in the AID determination instead of the total collection method used in faecal determination. In our study, AIA was used as a marker in both methods. The possible source of error and cause of variation in the AID determination may have been the sampling technique, i.e. manually squeezing the digesta out of the terminal ileum, which may have sucked in sloughed intestinal epithelial cells and other endogenous material in varying amounts. In addition, the AID method resulted in only one sample per animal, whereas in the ATTD method the sample was collected over 3 days, which may have decreased the accuracy of the AID samples.

Microbial fermentation in the large intestine converts both quantity and quality of the protein and AAs in the faeces. However, carnivores are considered to have low microbial fermentation capacity, because their digestive tract is short, the digesta passage rate is rapid and the colon is unsacculated (Szymeczko & Skrede 1990, Ahlstrøm & Skrede 1995, Tjernsbekk et al. 2014). Previous studies in carnivores have shown that low fermentation capacity is most pronounced in mink (Tjernsbekk et al. 2014, Gugołek et al. 2015). Blue foxes and dogs tend to have higher microbial activity (Hendriks et al. 2013, Tjernsbekk et al. 2014, Gugołek et al. 2015). In our study, the ATTD and AID methods resulted in diverging digestibility values among AAs. Arg, His, Cys, Asp, Gly, Pro, Ser and total AAs showed higher ATTD than AID coefficients, while Ile, Lys, Met, Ala and Tyr showed higher AID than ATTD coefficients. In previous studies, similar patterns of

AAs have shown higher or lower digestibility values using ATTD or AID measuring indicating AAs undergoing similar microbial fermentation in the large intestine of dogs and blue foxes (Szymeczko 2001, Hendriks & Sritharan 2002, Vhile et al. 2005, Tjernsbekk et al. 2014). In addition, net synthesis of Met in the large intestine has been demonstrated in swine and dog, which may lead to underestimation of Met digestibility (Mosenthin et al. 2000, Hendriks et al. 2012).

In the present study, more than half of the AAs showed lower AID than ATTD of AAs, which reflects a net loss of these AAs (Arg, Cys, His, Asp, Gly, Pro, Ser and total AAs) in the large intestine. Similar results were found in the study of Vhile et al. (2005) for Thr, Asp, Gly, Pro and Ser and for all AAs in the study of Szymeczko (2001). AAs disappearing from the large intestine are not considered to have nutritional value, since the fate of these AAs is to undergo microbial fermentation, and the possible absorption of nonprotein N does not contribute markedly to the protein metabolism of the animal. Thus, overestimation of the ATTD of these AAs is likely and must be taken into account when applying ATTD method to determine AA digestibility in blue foxes.

In dogs, the ATTD method has resulted in both over- and underestimation of digestibility in DM, protein and AAs (Muir et al. 1996, Murray et al. 1997, Hendriks & Sritharan 2002), and the AID method has been considered more accurate for the real AA availability, whereas in blue foxes the findings have been more unresolved. In blue foxes, previous authors have concluded that the difference between the AID and ATTD methods is numerically small, and the ATTD method may therefore be acceptable (Szymeczko 2001, Vhile et al. 2005, Tjernsbekk et al. 2014). Results obtained from our study support this approach. However, overestimation in the digestibility of Arg, Cys, His, Asp, Gly, Pro, Ser and total AAs and underestimation in digestibility, especially of Met,

389 Ile, Lys, Ala and Tyr must be taken into account. In addition, endogenous and microbial proteins
390 may affect the ATTD values and show ostensible reduction in the ATTD of AAs.
391
392 N excretion decreased with decreasing protein level, as was expected. The reduction in N excretion
393 with reduced protein supply has been clearly demonstrated in various species, including blue foxes
394 (Canh et al. 1998, Noblet et al. 2001, Dahlman et al. 2002a, Otto et al. 2003, Carpenter et al. 2004.
395 Belloir et al. 2017). In addition, N retention remained constant and, therefore, N utilisation
396 improved in low-protein diets. In a previous study in blue foxes, a similar effect was found
397 (Dahlman et al. 2002a). Corresponding to the study by Dahlman et al. (2002a), neither Met nor His
398 improved N utilisation, indicating that there was no deficiency of these AAs. However, the lowest
399 protein level was not tested without Met supplementation in our study.
400
401 The plasma urea (or plasma urea nitrogen, PUN) concentration is a rapid method for estimating AA
402 requirements and is affected by the quality and quantity of dietary protein and AA composition
403 (Coma 1995, Pedersen & Boisen 2001). The quality of the protein inversely affects the PUN, and
404 when the AA requirement of the animal is fulfilled, the need for cycling excess N to urea is
405 minimal, and the PUN reaches a minimum plateau (Coma 1995). However, in addition to quality,
406 the quantity of the dietary protein affects the PUN values directly. In addition, factors not related to
407 dietary protein, such as renal function, water intake and body protein catabolism under acute
408 challenges to the immune system, affect PUN values (Coma 1995, Kiarie et al. 2009). In studies in
409 swine, N retention and PUN values have shown similar responses in determining the AA
410 requirement of the animal (Coma et al. 1995, Pedersen et al. 2003). Correspondingly, the absolute
411 plasma urea in our study was lowest in group P16M, which showed the highest N retention. This
412 supports the conclusions obtained from the N retention results that excess N is mainly excreted in
413 the urine. Still, plasma urea did not differ between diets. Varying protein contents of the diets may

414 have affected to plasma urea values. In previous studies, plasma urea reference value for healthy
415 blue foxes, fed normal diets based on recommendations, has been 6.4 mmol/l, while for breeding
416 blue fox females, the PUN has ranged from 6.18 to 6.67 mmol/l (Korhonen & Huuki 2014,
417 Sepponen et al. 2014). Our result for plasma urea from 4.92 to 5.96 mmol/l was lower, probably
418 due to lower dietary protein level than in normal farm diets. However, PUN was determined in
419 fasted state, which decreases the effect of
420
421 Despite the fairly constant digestibility coefficients and improved N utilisation results, decreased
422 plasma AAs of some AAs were measured in low-protein diets. The decreased plasma AAs may
423 indicate that the low-protein diets did not provide adequate AA to cover to requirement, especially
424 if the feeding period would have been extended. In dogs, acute protein deficiency results in
425 decreasing plasma AAs in all AAs. In long-term protein deficiency, however, only essential AAs in
426 plasma decrease, while nonessential tend to increase (NRC 2006). Our study lasted only 7 days, but
427 it may be noteworthy that essential AAs in plasma decreased with decreasing protein level. Met and
428 His supplementation probably influenced the concentration of these AAs in plasma, since the Met
429 and His plasma concentrations were constant between the control and in the supplemented low-
430 protein groups. In previous studies in pigs, supplemented AAs have markedly increased the plasma
431 content of the supplemented AAs in question (e.g. Figueroa et al. 2003). The plasma AA profile of
432 blue foxes has not been determined. In comparison to the plasma AA content of food-deprived (24
433 h) dogs, most of the AA concentrations in our study were similar or higher, except Cys and Thr,
434 which were lower and may reflect the importance of these AAs for blue foxes (Delaney et al. 2001).
435

436 **5. Conclusions**

437 In the present study, the ATTD of CP decreased with decreasing dietary protein. The effect of
438 greater proportions of endogenous protein in low-protein diets supposedly affected the decreasing

apparent digestibility. In addition, we concluded that the wheat starch added in the low-protein diets contributed the increased ATTD of DM, OM and CCH. The supplemented Met and His were well digested and increased the Met and His digestibility, respectively, but did not improve the overall digestibility nor affected any of the other variables examined.

The ATTD method showed the decreasing digestibility of the nonessential and some essential AAs with decreasing protein level. The AID method varied more between individuals and failed to show the differences between the diets. However, the ATTD method may have resulted in overestimation of the digestibility of Arg, Cys, His, Asp, Gly, Pro, Ser and total AAs and underestimation of the digestibility of Ile, Lys, Met, Ala and Tyr. According to this study, ATTD can be applied in determining AAs digestibility in blue fox as it is less invasive and more convenient method. However, the differences in single AAs must be taken into account when estimating the true AA digestibility.

N retention did not differ among the diets. N excretion decreased and N utilisation improved with lower dietary protein supplies. Therefore, we concluded that the protein supply and AA composition in the low-protein diet with supplemented Met was adequate for blue foxes in the late growing-furring season. According to this study, digestible Met level of 0.32g/MJ ME. However, His supplementation was inadequate compared with the design, and it is therefore impossible to draw conclusions of the importance of His supplementation.

Acknowledgements

The authors would like to thank the staff at the Kannus Research Farm Luova Oy for technical assistance and care of the experimental animals, and laboratory personnel at the University of Helsinki and Fin Furlab for carrying out the feed analyses. Essi Tuomola is acknowledged for formulating the diets used in the study. The study was supported by the Helve Foundation. The funding source had no involvement in the study design, conduction of the experiments, analysis and interpretation of the data, writing of the article or the decision to submit the article for publication.

Conflict of interest

Vappu Ylinen received a personal research grant from the Helve Foundation for conducting the study. At the time the research was done, Päivi Pylkkö worked for Kannus Research Farm Luova Oy. Jussi Peura is the Research Director in Finnish Fur Breeders' Association. The above-mentioned authors declare no financial interest or benefit from the direct applications of the research. Jarmo Valaja declares that he has no conflict of interest, financial interest or benefit from the direct applications of the research.

Use of animals

All institutional and national guidelines for the care and use of experimental animals were followed. All the experimental procedures were approved by the National Animal Experiment Board in Finland with the guidelines established by the European Union Directive 2010/63/EU and current Finnish legislation on animal experimentation (Act on the Protection of Animals Used for Scientific or Educational Purposes 497/2013).

Data availability

The data that support the findings of this study are available from the corresponding author [V. Y.] upon reasonable request.

487 Table 1. Composition of the experimental diets (g/kg) and planned chemical composition.

	P24	P20	P20M	P16M	P16MH
Baltic herring, autumn	170	140	140	108	108
Slaughter by-products, pork	30	25	25	19	19
Slaughter by-products, broiler	260	213	213	165	165
Precooked barley	170	139	139	107	107
Animal protein, meal	21	15	15	11	11
Fish meal	36	29	29	23	23
Molasses from sugar beet pulp	20	25	25	26	26
Wheat bran	20	25	25	26	26
Lard	56	58	58	75	75
Mineral mixture[a]	2	2	2	2	2
Cooked wheat starch		54	54	78	78
DL-methionine			0.8	1.3	1.3
L-histidine					0.9
Celite	6	6	6	6	6
Water	209	269	268	353	352
Planned in DM (g/kg)					
Protein	291	248	249	212	214
Fat	277	258	257	285	284
Carbohydrates	363	453	453	479	478
Ash	68.3	58.2	58.1	49.9	49.8
ME (MJ ME/kg DM)	18.7	18.8	18.8	19.8	19.8
Energy distribution					
Protein	24	20	20.1	16	16.2
Fat	55	50.7	50.7	53.3	53.1
Carbohydrates	21	29.3	29.3	30.7	30.6
F:C	2.6	1.7	1.7	1.7	1.7

488 P24 = crude protein 24% of ME, P20 = crude protein 20% of ME, P20M =

489 crude protein 20% of ME + Met supplement, P16M = crude protein 16% of ME

490 + Met supplement, P16MH = crude protein 16% of ME + Met and His supplements. ME =

491 metabolizable energy, Met = DL-methionine, His = L-histidine.

492 [a] Containing 100 g1 mixture—Ca: 16 g; P: 11 g; Mg: 4 g; Fe: 650 mg; Zn: 600 mg; Mn: 300 mg;

493 Cu: 15 mg; Co: 4 mg; Se: 0.1 mg.

494

495 Table 2. Analysed chemical composition of the diets.

	P24	P20	P20M	P16M	P16MH
DM g/kg	398	371	361	371	371
In DM (g/kg DM)					
Protein	278	246	242	229	207
Fat	256	300	321	274	254
Carbohydrates	384	375	358	423	469
Ash	82	79	79	74	70
ME (MJ/kg DM)	18.2	18.4	19.5	19.0	18.4
Energy distribution*					
Protein	21.3	17.1	16.4	15.9	14.1
Fat	52.8	60.3	62.6	55.1	51.6
Carbohydrates	25.9	22.5	21.0	29.0	34.3
F:C	2.0	2.7	3.0	1.9	1.5

496 P24 = crude protein 24% of ME, P20 = crude protein 20% of ME, P20M

497 = crude protein 20% of ME + Met supplement, P16M = crude protein

498 16% of ME + Met supplement, P16MH = crude protein 16% of ME +

499 Met and His supplements. DM = dry matter, ME = metabolizable energy,

500 F:C = fat:carbohydrate ratio, Met = DL-methionine, His = L-histidine.

501 * Energy distribution as % of ME, calculated using chemical composition

502 and digestibility coefficients carried out with the feed in question.

503

504 Table 3. Analysed amino-acid composition of the diets (g/kg DM).

	P24	P20	P20M	P16M	P16MH
Essential AA + cysteine					
Arginine	16.9	14.6	15.1	14.2	12.6
Cysteine	3.8	2.7	3.3	3.6	2.7
Histidine	6.3	5.2	5.3	5.2	5.6
Isoleucine	10.2	9.5	9.8	9.0	8.2
Leucine	20.6	17.8	18.0	17.4	15.1
Lysine	15.3	14.2	14.1	14.0	11.8
Methionine	5.1	4.0	6.4	7.1	6.9
Phenylalanine	11.9	10.0	10.3	9.7	8.7
Threonine	10.8	9.7	9.8	9.4	8.3
Tryptophan	1.9	1.7	1.7	1.5	1.5
Valine	14.7	12.8	12.9	12.5	10.8
Nonessential AA					
Alanine	16.4	14.2	14.3	14.0	11.9
Aspartic acid	22.4	20.1	20.2	19.8	16.9
Glutamic acid	38.2	34.5	34.3	32.9	28.7
Glycine	20.3	16.9	17.2	16.6	14.5
Proline	17.2	14.6	14.5	14.0	12.0
Serine	13.4	11.7	11.9	11.4	10.0
Tyrosine	7.9	6.8	7.3	6.7	6.1
Σ Essential[a]	117	102	107	104	92
Σ Nonessential[a]	136	119	120	115	100
Σ Total[b]	253	221	226	219	192

505 [a]As listed in the table, [b]Total = Σ Essential + Σ Nonessential.

506 P24 = crude protein 24% of ME, P20 = crude protein 20% of ME, P20M = crude

507 protein 20% of ME + Met supplement, P16M = crude protein 16% of ME

508 + Met supplement, P16MH = crude protein 16% of ME + Met and His supplements. AA = amino

509 acid, ME = metabolizable energy, Met = DL-methionine, His = L-histidine.

510 Table 4. Apparent total-tract digestibility of macronutrients.

	P24	P20	P20M	P16M	P16MH	SEM[1]	c1	c2	c3	c4
DM	72.2	70.3	71.3	73.7	74.4	0.748	ns	**	ns	ns
CP	74.0	70.1	71.5	70.8	68.1	1.136	*	ns	ns	ns
OM	77.8	76.0	77.4	79.5	79.7	0.774	ns	**	ns	ns
CCH	69.7	64.5	65.1	73.6	76.8	1.439	ns	***	ns	ns
CF	94.1	95.3	95.6	95.8	94.3	0.360	*	ns	ns	*
Ash	9.08	3.48	0.79	1.75	4.92	1.327	***	ns	ns	ns

511 P24 = crude protein 24% of ME, P20 = crude protein 20% of ME, P20M = crude protein 20% of
512 ME + Met supplement, P16M = crude protein 16% of ME + Met supplement, P16MH = crude
513 protein 16% of ME + Met and His supplements. DM = dry matter, CP = crude protein, OM =
514 organic matter, CCH = crude carbohydrates, CF = crude fat. SEM = standard error of the means.
515 SEM[1] is for diets P24, P20 and P16M. SEM for diets P20M and P16MH is proportionately 1.154
516 of the reported value. ns = nonsignificant, * = significance at $p < 0.05$, ** = significance at $p <$
517 0.01, *** = significance at $p < 0.001$. ME = metabolizable energy, Met = DL-methionine, His =
518 L-histidine. Contrasts: C1 = P24 vs. others, C2 = protein level (P20 and P20M vs. P16M and
519 P16MH), C3 = MET-supplementation (P20 vs. P20M), C4 = HIS-supplementation (P16M vs.
520 P16MH).

521 Table 5. Apparent ileal digestibility of amino acids.

	P24	P20	P20M	P16M	P16MH	SEM	c1	c2	c3	c4
Essential AA + cysteine										
Arginine	77.7	77.1	77.7	77.9	77.6	1.096	ns	ns	ns	ns
Cysteine	37.2	14.6	32.4	42.0	23.8	4.844	ns	ns	*	*
Histidine	77.0	77.6	78.5	77.9	82.2	1.391	ns	ns	ns	*
Isoleucine	75.9	75.2	75.5	73.3	75.6	1.375	ns	ns	ns	ns
Leucine	76.1	76.4	77.0	76.0	76.7	1.278	ns	ns	ns	ns
Lysine	81.5	83.7	84.9	84.7	83.8	1.162	ns	ns	ns	ns
Methionine	86.6	84.2	90.4	90.3	91.6	1.020	*	**	***	ns
Phenylalanine	75.5	74.6	76.3	75.2	75.8	1.416	ns	ns	ns	ns
Threonine	67.6	65.4	66.1	63.2	63.8	2.067	ns	ns	ns	ns
Tryptophan	63.7	60.3	61.5	54.5	57.9	2.346	ns	ns	ns	ns
Valine	72.4	72.0	72.1	71.0	71.8	1.508	ns	ns	ns	ns
Nonessential AA[a]										
Alanine	76.8	77.5	77.4	77.4	76.0	1.103	ns	ns	ns	ns
Aspartic acid	63.8	63.9	64.9	63.1	65.3	2.212	ns	ns	ns	ns
Glutamic acid	77.7	77.3	77.1	76.7	77.2	1.355	ns	ns	ns	ns
Glycine	68.8	67.4	67.7	67.9	67.1	1.522	ns	ns	ns	ns
Proline	66.6	64.2	63.6	63.2	61.2	1.768	ns	ns	ns	ns
Serine	62.6	60.1	60.2	57.4	58.9	2.214	ns	ns	ns	ns
Tyrosine	75.7	73.3	75.2	74.7	77.2	1.650	ns	ns	ns	ns
Total[b]	73.6	73.0	73.8	73.0	73.6	1.419	ns	ns	ns	ns

522 [a]As listed in the table, [b]Total = Σ Essential + Σ Nonessential. P24 = crude protein 24% of ME, P20
523 = crude protein 20% of ME, P20M = crude protein 20% of ME + Met supplement, P16M = crude
524 protein 16% of ME + Met supplement, P16MH = crude protein 16% of ME + Met and His
525 supplements. SEM = standard error of the means. ns = nonsignificant, * = significance at $p < 0.05$,
526 ** = significance at $p < 0.01$, *** = significance at $p < 0.001$. AA = amino acid, ME =
527 metabolizable energy, Met = DL-methionine, His = L-histidine. Contrasts: C1 = P24 vs. others, C2

528 = protein level (P20 and P20M vs. P16M and P16MH), C3 = MET-supplementation (P20 vs.

529 P20M), C4 = HIS-supplementation (P16M vs. P16MH).

530

Table 6. Apparent total-tract digestibility of amino acids.

	P24	P20	P20M	P16M	P16MH	SEM	c1	c2	c3	c4
Essential AA + cysteine										
Arginine	81.7	79.5	80.1	82.5	79.8	0.789	ns	ns	ns	*
Cysteine	50.8	32.0	48.7	52.1	43.2	4.113	ns	ns	*	ns
Histidine	81.8	79.5	81.2	83.3	84.3	0.853	ns	**	ns	ns
Isoleucine	73.2	70.5	72.9	73.4	70.7	0.991	ns	ns	ns	ns
Leucine	76.3	74.0	75.7	77.5	74.0	0.766	ns	ns	ns	**
Lysine	81.2	81.3	81.9	84.6	79.5	0.988	ns	ns	ns	**
Methionine	78.1	73.7	84.8	86.5	87.3	1.964	*	**	**	ns
Phenylalanine	75.7	72.0	74.1	76.8	73.2	1.114	ns	ns	ns	*
Threonine	70.8	65.0	67.6	68.6	64.4	1.180	**	ns	ns	*
Tryptophan	68.2	61.8	60.7	60.6	60.9	1.943	**	ns	ns	ns
Valine	72.6	69.2	71.0	73.0	68.9	0.875	*	ns	ns	**
Nonessential AA[a]										
Alanine	75.7	71.7	72.7	74.1	69.5	1.035	**	ns	ns	**
Aspartic acid	72.3	68.8	68.7	69.0	64.0	0.826	***	*	ns	***
Glutamic acid	79.3	76.4	76.4	76.6	73.3	0.639	***	*	ns	**
Glycine	79.4	75.0	75.6	78.9	76.0	1.141	*	ns	ns	ns
Proline	75.3	70.7	70.8	74.2	69.9	1.125	**	ns	ns	*
Serine	69.3	63.7	64.8	68.1	63.3	1.158	**	ns	ns	*
Tyrosine	71.9	67.6	70.9	72.5	68.3	1.432	ns	ns	ns	ns
Total[b]	77.2	73.9	75.2	77.0	73.5	0.751	*	ns	ns	**

[a]As listed in the table, [b]Total = Σ Essential + Σ Nonessential. P24 = crude protein 24% of ME, P20 = crude protein 20% of ME, P20M = crude protein 20% of ME + Met supplement, P16M = crude protein 16% of ME + Met supplement, P16MH = crude protein 16% of ME + Met and His supplements. SEM = standard error of the means. ns = nonsignificant, * = significance at $p < 0.05$, ** = significance at $p < 0.01$, *** = significance at $p < 0.001$. AA = amino acid, ME = metabolizable energy, Met = DL-methionine, His = L-histidine. Contrasts: C1 = P24 vs. others, C2

538 = protein level (P20 and P20M vs. P16M and P16MH), C3 = MET-supplementation (P20 vs.

539 P20M), C4 = HIS-supplementation (P16M vs. P16MH).

540

Table 7. Average apparent ileal and total-tract digestibility of amino acids

	Ileal	Total-tract	SEM	Method	Diet*method
Essential AA + cystine					
Arginine	77.6	80.7	0.426	***	ns
Cysteine	30.0	45.4	2.666	***	ns
Histidine	78.6	82.0	0.613	***	ns
Isoleucine	75.1	72.2	0.540	***	ns
Leucine	76.4	75.5	0.479	ns	ns
Lysine	83.7	81.7	0.542	*	ns
Methionine	88.6	82.1	1.150	***	ns
Phenylalanine	75.5	74.4	0.585	ns	ns
Threonine	65.2	67.3	0.812	ns	ns
Tryptophan	59.6	62.4	1.105	ns	ns
Valine	71.8	70.9	0.567	ns	ns
Nonessential AA[a]					
Alanine	77.0	72.7	0.554	***	ns
Aspartic acid	64.2	68.6	0.800	***	ns
Glutamic acid	77.2	76.4	0.526	ns	ns
Glycine	67.8	77.0	0.619	***	ns
Proline	63.8	72.2	0.741	***	ns
Serine	59.8	65.9	0.850	***	ns
Tyrosine	75.2	70.2	0.722	***	ns
Total[b]	73.4	75.4	0.517	*	ns

[a]As listed in the table, [b]Total = Σ Essential + Σ Nonessential.

ns = nonsignificant, * = significance at $p < 0.05$,** = significance at $p < 0.01$,

*** = significance at p <0 .001. SEM = standard error of the means, AA =

amino acid.

549 Table 8. Nitrogen (N) metabolism.

	P24	P20	P20M	P16M	P16MH	SEM[1]	c1	c2	c3	c4
N intake	13.2	11.7	11.5	10.9	9.8					
Faecal N g/d	3.2	3.1	3.0	3.1	2.9	0.137	ns	ns	ns	ns
UrinaryN g/d	8.4	7.4	7.0	6.2	5.6	0.291	***	***	ns	ns
Urea g/d	16.1	12.9	13.0	12.0	10.5	0.564	***	*	ns	ns
Urea N g/d	7.5	6.0	6.4	5.6	4.9	0.263	***	*	ns	ns
Urea N as % of total urinary N	89.0	81.6	86.4	91.2	87.4	2.464	ns	ns	ns	ns
N absorbed g/d	10.0	8.6	8.5	7.8	6.9	0.137	***	***	ns	**
N retained										
g per day/animal	1.5	1.2	1.3	1.6	1.3	0.313	ns	ns	ns	ns
% of intake	11.5	10.5	11.7	14.7	13.7	2.977	ns	ns	ns	ns
g kg live weight	0.1	0.1	0.1	0.1	0.1	0.020	ns	ns	ns	ns
% of absorption	15.2	14.4	15.8	20.3	19.0	4.100	ns	ns	ns	ns
Plasma urea mmol/l	5.96	5.52	4.98	4.92	5.54	0.424	ns	ns	ns	ns

550 P24 = crude protein 24% of ME, P20 = crude protein 20% of ME, P20M = crude protein 20% of
551 ME + Met supplement, P16M = crude protein 16% of ME + Met supplement, P16MH = crude
552 protein 16% of ME + Met and His supplements. PUN = plasma urea nitrogen. SEM = standard
553 error of the means. SEM[1] is for diets P24, P20 and P16M. SEM for diets P20M and P16MH is
554 proportionately 1.154 of the reported value. ns = nonsignificant, * = significance at $p < 0.05$, ** =
555 significance at $p < 0.01$, *** = significance at $p < 0.001$. ME = metabolizable energy, Met = DL-
556 methionine, His = L-histidine. Contrasts: C1 = P24 vs. others, C2 = protein level (P20 and P20M
557 vs. P16M and P16MH), C3 = MET-supplementation (P20 vs. P20M), C4 = HIS-supplementation
558 (P16M vs. P16MH).
559
560

561 Table 9. Plasma amino acids μM/l.

	P24	P20	P20M	P16M	P16MH	SEM	c1	c2	c3	c4
Essential AA + cystine										
Arginine	148.0	148.7	153.8	148.6	157.0	12.329	ns	ns	ns	ns
Cysteine	34.63	36.03	37.20	37.88	39.95	3.5803	ns	ns	ns	ns
Histidine	76.14	70.33	72.06	76.65	71.42	2.6257	ns	ns	ns	ns
Isoleucine	79.92	72.69	75.63	65.54	65.43	4.0402	*	*	ns	ns
Leucine	161.1	150.1	150.5	134.8	135.1	6.4887	*	*	ns	ns
Lysine	185.4	175.2	190.1	190.1	168.8	11.386	ns	ns	ns	ns
Methionine	44.06	42.57	42.35	39.75	42.64	2.4933	ns	ns	ns	ns
Phenylalanine	94.54	85.09	87.47	85.90	79.72	3.8300	*	ns	ns	ns
Threonine	120.6	109.5	104.4	95.28	90.39	5.3046	**	*	ns	ns
Tryptophan	51.71	45.36	39.84	41.35	39.35	2.6993	**	ns	ns	ns
Valine	196.0	181.2	182.7	168.0	165.4	7.8989	*	ns	ns	ns
Nonessential AA										
Alanine	415.3	449.1	469.2	535.7	471.2	38.956	ns	ns	ns	ns
Aspartic acid	73.04	64.90	67.44	62.25	60.67	2.6611	**	ns	ns	ns
Glutamic acid	850.0	733.8	703.1	606.2	667.3	78.765	ns	ns	ns	ns
Glycine	305.3	261.6	294.3	282.2	284.7	13.553	ns	ns	ns	ns
Proline	135.8	133.7	135.0	142.9	139.1	8.1806	ns	ns	ns	ns
Serine	234.0	241.5	232.6	212.9	208.4	12.809	ns	ns	ns	ns
Tyrosine	60.74	54.32	53.37	52.51	47.11	3.6975	*	ns	ns	ns

562 P24 = crude protein 24% of ME, P20 = crude protein 20% of ME, P20M = crude protein 20% of
563 ME + Met supplement, P16M = crude protein 16% of ME + Met supplement, P16MH = crude
564 protein 16% of ME + Met and His supplements, SEM = standard error of the means.
565 ns = nonsignificant, * = significance at $p < 0.05$, ** = significance at $p < 0.01$, *** = significance at
566 $p < 0.001$. AA = amino acid, ME = metabolizable energy, Met = DL-methionine, His = L-histidine.
567 Contrasts: C1 = P24 vs. others, C2 = protein level (P20 and P20M vs. P16M and P16MH), C3 =
568 MET-supplementation (P20 vs. P20M), C4 = HIS-supplementation (P16M vs. P16MH).

References

Aarnink, A. J. A., & Verstegen, M. W. A. (2007). Nutrition, key factor to reduce environmental load from pig production. Livestock Science, 109, 194–203. https://doi.org/10.1016/j.livsci.2007.01.112

Adeola, O., Xue, P. C., Cowieson, A. J., & Ajuwon, K. M. (2016). Basal endogenous losses of amino acids in protein nutrition research for swine and poultry. Animal Feed Science and Technology, 221, 274–283. https://doi.org/10.1016/j.anifeedsci.2016.06.004

Ahlstrøm, Ø., & Skrede, A. (1995). Comparative nutrient digestibility in blue foxes (Alopex lagopus) and mink (Mustela vison) fed diets with diverging fat: Carbohydrate ratios. Acta Agriculturae Scandinavica A: Animal Sciences, 45, 74–80. https://doi.org/10.1080/09064709509410916

Belloir, P., Méda, B., Lambert, W., Corrent, E., Juin, H., Lessire, M., & Tesseraud, S. (2017). Reducing the CP content in broiler feeds: impact on animal performance, meat quality and nitrogen utilisation. Animal, 11, 1881–1889. doi:10.1017/S1751731117000660

Canh, T. T., Aarnink, A. J. A., Schutte, J. B., Sutton, A., Langhout, D. J., & Verstegen, M. W. A. (1998). Dietary protein affects nitrogen excretion and ammonia emission from slurry of growing-finishing pigs. Livestock Production Science, 56, 181–191. https://doi.org/10.1016/S0301-6226(98)00156-0

Carpenter, D. A., O'Mara, F. P., & O'Doherty, J. V. (2004). The effect of dietary crude protein concentration on growth performance, carcass composition and nitrogen excretion in entire grower-finisher pigs. Irish Journal of Agricultural and Food Research, 43, 227–236.

590 Coma, J. (1995). "Use of plasma urea nitrogen as a rapid response criterion to estimate the lysine
591 requirements of growing and lactating pigs ". Retrospective Theses and Dissertations 10892.
592 https://lib.dr.iastate.edu/rtd/10892

593 Coma, J., Carrion, D., & Zimmerman, D. R. (1995). Use of plasma urea nitrogen as a rapid
594 response criterion to determine the lysine requirement of pigs. Journal of Animal Science, 73, 472–
595 481.

596 Dahlman, T., Kiiskinen, T., Mäkelä, J., Niemelä, P., Syrjälä-Qvist, L., Valaja, J., & Jalava, T.
597 (2002a). Digestibility and nitrogen utilisation of diets containing protein at different levels and
598 supplemented with DL-methionine, L-methionine and L-lysine in blue fox (Alopex lagopus).
599 Animal Feed Science and Technology, 98, 219–235.

600 Dahlman, T., Mäntysalo, M., Rasmussen P.V., & Skovløkke, L. L. (2002b). Influence of dietary
601 protein level and the amino acids methionine and lysine on leather properties of blue fox (Alopex
602 lagopus) pelts. Archives of Animal Nutrition, 56, 443–454.

603 Dahlman, T. (2003). Protein and Amino Acids in the Nutrition of the Growing-Furring Blue Fox.
604 University of Helsinki. Department of Animal Science Publications. Helsinki. 33 p.

605 Dahlman, T., Valaja, J., Venäläinen, E., Jalava, T., & Pölönen, I. (2004). Optimum dietary amino
606 acid pattern and limiting order of some essential amino acids for growing-furring blue foxes
607 (Alopex lagopus). Animal Science, 78, 77–86.

608 Delaney, S. J., Hill, A. S., Backus, R. C., Czarnecki-Maulden, G. L. & Rogers, Q. R. (2001).
609 Dietary crude protein concentration does not affect the leucine requirement of growing dogs.
610 Journal of Animal Physiology and Animal Nutrition, 85, 88–100.

611 European Commission. 1998. Commission Directive 98/64/EC. Community methods of analysis
612 for the determination of amino acids, crude oils and fats, and olaquindox in feedingstuffs and
613 amending directive 71/393/EEC. Official Journal of European Communities, L 257, 4–23.

614 Fan, M. Z., & Sauer, W. C. (1997). Determination of true ileal amino acid digestibility in feedstuffs
615 for pigs with the linear relationships between distal ileal outputs and dietary inputs of amino acids.
616 Journal of the Science of Food and Agriculture, 73(2), 189–199.
617 https://doi.org/10.1002/(SICI)1097-0010(199702)73:2<189::AID-JSFA702>3.0.CO;2-N

618 Figueroa, J. L., Lewis, A. J., Miller, P. S., Fischer, R. L., & Diedrichsen, R. M. (2003). Growth,
619 carcass traits, and plasma amino acid concentrations of gilts fed low-protein diets supplemented
620 with amino acids including histidine, isoleucine and valine. Journal of Animal Science, 81, 1529–
621 1537.

622 Gugołek, A., Juśkiewicz, J., Strychalski, J., Konstantynowicz, M., & Zwoliński, C. (2015). Nutrient
623 digestibility and colonic fermentation processes in species of the families Mustelidae and Canidae
624 fed the same diet. Journal of Experimental Zoology Part A: Ecological Genetics and Physiology,
625 323, 637–644. https://doi.org/10.1002/jez.1954

626 Gugołek, A., Wyczling, T., Strychalski, J., Kowalska, D., Konstantynowicz, M., & Zwoliński, C.
627 (2017). Effect of diets with high methionine levels on growth performance, health status, nutrient
628 digestibility and nitrogen retention in arctic foxes. Journal of Animal and Plant Sciences, 27, 1743–
629 1749.

630 Hansen, N.E., Finne, L. Skrede, A. & Tauson, A.-H. 1991. Energiforsyningen hos mink ag ræv.
631 Nordic Association of Agricultural Scientist, Nordic Association of Agricultural Scientists.
632 Subsection for Fur Animals. Report No. 63. 59 p.

633 Hendriks, W. H., & Sritharan, K. (2002). Apparent ileal and fecal digestibility of dietary protein is
634 different in dogs. The Journal of Nutrition, 132, 1692S–1694S.

635 Hendriks, W. H., Van Baal, J., & Bosch, G. (2012). Ileal and faecal protein digestibility
636 measurement in humans and other non-ruminants - A comparative species view. British Journal of
637 Nutrition, 108, S247–S257. https://doi.org/10.1017/S0007114512002395.

638 Hendriks, W. H., Thomas, D. G., Bosch, G., & Fahey, G. C. (2013). Comparison of ileal and total
639 tract nutrient digestibility of dry dog foods. Journal of Animal Science, 91, 3807–3814.
640 https://doi.org/10.2527/jas.2012-5864

641 Kiarie, E., Slominski, B. A., Krause, D. O., & Nyachoti C. M. (2009). Acute phase response of
642 piglets fed diets containing non-starch polysaccharide hydrolysis products and egg yolk antibodies
643 following an oral challenge with *Escherichia coli (k88)*. Canadian Journal of Animal Science, 89,
644 353–360. https://doi.org/10.4141/CJAS09008

645 Korhonen, H. T., & Huuki, H. (2014). Serum Biochemistry and Hematology in Blue Fox (Vulpes
646 lagopus). Open Journal of Veterinary Medicine, 04, 255–260.
647 https://doi.org/10.4236/ojvm.2014.411030

648 de Lange, C.F.M., Sauer, W. C., Mosenthin, R. & Souffrant, W. B. (1989). The Effect of Feeding
649 Different Protein-Free Diets on the Recovery and Amino Acid Composition of Endogenous Protein
650 Collected from the Distal Ileum and Feces in Pigs. Journal of Animal Science, 67, 746–754.

651 Lemme, A., Ravindran, V., & Bryden, W. L. (2004). Ileal digestibility of amino acids in feed
652 ingredients for broilers. World's Poultry Science Journal, 60, 423–438.
653 doi:http://dx.doi.org/10.1079/WPS200426

654 Low, A. G. (1980). Nutrient Absorption in Pigs. Journal of the Science of Food and Agriculture, 31,
655 1087–1130.

LUKE (2006). The Feed Tables and Feeding Recommendations 2006. MTT:n Selvityksiä 106. MTT, Jokioinen. https://portal.mtt.fi/portal/page/portal/Rehutaulukot/feed_tables_english/feed_tables/fur_animals

McDonald, P., Edwards, R.A., Greenhalgh, J.F.D., Morgan, C.A., Sinclair, L.A. and Wilkinson, R.G. (2011) Animal Nutrition. 7th Edition. Pearson Education Limited, Essex, England.

Mosenthin, R., Sauer, W. C., Blank, R., Huisman, J., & Fan, M. Z. (2000). The concept of digestible amino acids in diet formulation for pigs. Livestock Production Science, 64, 265–280. https://doi.org/10.1016/S0301-6226(99)00139-6

Muir, H. E., Murray, S. M., Fahey, Jr., G. C., Merchen, N. R., & Reinhart, G. A. (1996). Nutrient Digestion by Ileal Cannulated Dogs as Affected by Dietary Fibers with Various Fermentation Characteristics. Journal of Animal Sciences, 74, 1641–1648.

Murray, S. M., Patil, A. R., Fahey, Jr., G. C., Merchen, N. R., & Hughes, D. M. (1997). Raw and Rendered Animal By-Products as Ingredients in Dog Diets. Journal of Animals Sciences, 75, 2497–2505.

NRC (2006). *Nutrient Requirements of Dogs and Cats*. National Research Council. Washington, DC: The National Academies Press. https://doi.org/10.17226/10668.

Noblet, J., Le Bellego, L., Van Milgen, J., & Dubois, S. (2001). Effects of reduced dietary protein level and fat addition on heat production and nitrogen and energy balance in growing pigs. Animal Research, 50, 227–238. https://doi.org/10.1051/animres:2001129

Otto, E. R., Yokoyama, M., Ku, P. K., Ames, N. K., & Trottier, N. L. (2003). Nitrogen balance and ileal amino acid digestibility in growing pigs fed diets reduced in protein concentration. Journal of Animal Sciences, 81, 1743–1753. https://doi.org/10.2527/2003.8171743x

678 Pedersen, C., & Boisen, S. (2001). Studies on the Response Time for Plasma Urea Nitrogen as a
679 Rapid Measure for Dietary Protein Quality in Pigs, Acta Agriculturae Scandinavica, Section A —
680 Animal Science, 51, 209–216. https://doi.org/10.1080/09064700152717182

681 Pedersen, C., Lindberg, J. E., & Boisen ,S. (2003). Determination of the optimal dietary
682 threonine:lysine ratio for finishing pigs using three different methods. Livestock Production
683 Science, 82, 233–243. https://doi.org/10.1016/S0301-6226(03)00011-3.

684 Perttilä, S., Valaja, J., Partanen, K., Jalava, T., & Venäläinen, E. (2002). Apparent ileal digestibility
685 of amino acids in protein feedstuffs and diet formulation based on total vs digestible lysine for
686 poultry. Animal Feed Science and Technology, 98, 203–218. https://doi.org/10.1016/S0377-
687 8401(02)00031-7

688 Profur 2019. Toimintakäsikirja 2018 – 2019 (in Finnish). Suomen Turkiseläin Kasvattajain Liitto
689 ry. Vantaa.

690 Puhakka, L., Jaakkola, S., Simpura, I., Kokkonen, T., & Vanhatalo, A. (2016). Effects of replacing
691 rapeseed meal with fava bean at 2 concentrate crude protein levels on feed intake, nutrient
692 digestion, and milk production in cows fed grass silage–based diets. Journal of Dairy Science, 99,
693 7993–8006.

694 Sauer, W. C., & Ozimek, L. (1986). Digestibility of amino acids in swine: results and their practical
695 applications. A review. Livestock Production Science, 15, 367–388.

696 Sauer, W. C., Mosenthin, R., & Hartog, L. a Den. (1991). The effect of source of fiber on ileal and
697 fecal amino acid digestibility and bacterial nitrogen excretion in growing pigs. Journal of Animal
698 Science, 69, 4070–4077.

699 Sauer WC, Fan MZ, Mosenthin R & Drochner W. (2000). Methods for measuring ileal amino acid
700 digestibility in pigs. In: D'Mello JPF (ed.): Farm Animal Metabolism and Nutrition. CABI
701 Publishing, pp. 279–306.

702 Sepponen, J., Korhonen, H. T., Eskeli, P., & Koskinen, N. (2014). Tuotanto- ja siitoskauden
703 ruokinnan vaikutus siniketun rasva-aineenvaihduntaan sekä siitoskuntoon. (In Finnish.) MTT
704 Loppuraportti. 13 p.

705 Silvio, J., Harmon, D. L., Gross, K. L., & McLeod, K. R. (2000). Influence of fiber fermentability
706 on nutrient digestion in the dog. Nutrition, 16, 289–295. https://doi.org/10.1016/S0899-
707 9007(99)00298-1

708 Skrede, A., Krogdahl, Å., & Austreng, E. (1980). Digestibility of amino acids in raw fish flesh and
709 meat- and- bone meal for the chicken, fox, mink and rainbow trout. Zeitschrift Für Tierphysiologie
710 Tierernährung Und Futtermittelkunde, 43, 92–101. https://doi.org/10.1111/j.1439-
711 0396.1980.tb00611.x

712 Szymeczko, R., & Skrede, A. (1990). Protein digestion in mink, Acta Agriculturae Scandinavica,
713 40, 189-200, DOI: 10.1080/00015129009438018

714 Szymeczko, R. (2001). Ileal and total digestibility of amino acids in feeds used in mink and polar
715 fox nutrition. Journal of Animal and Feed Sciences, 10, 211–222.

716 Tjernsbekk, M. T., Tauson A-H., & Ahlstrøm, Ø. (2014) Ileal, colonic and total tract nutrient
717 digestibility in dogs (*Canis familiaris*) compared with total tract digestibility in mink (*Neovison*
718 *vison*), Archives of Animal Nutrition, 68, 245-261, DOI: 10.1080/1745039X.2014.915137

719 Vhile, S. G., Skrede, A., Ahlstrom, O., Szymeczko, R., & Hove, K. (2005). Ileal and total tract
720 nutrient digestibility in blue foxes (Alopex lagopus) fed extruded diets containing different protein
721 sources. Archives of Animal Nutrition, 59, 61–72. https://doi.org/10.1080/17450390512331342395

722 Wang, T. C., & Fuller, M. F. (1989). The optimum dietary amino acid pattern for growing pigs.
723 British Journal of Nutrition, 62, 17-89.

724 Wenk C. (2001). The role of dietary fibre in the digestive physiology of the pig. Animal Feed
725 Science and Technology, 90, 21–33. doi: 10.1016/S0377-8401(01)00194-8.

726 Williams, P. E. V. (1995). Digestible amino acids for non-ruminant animals: theory and recent
727 challenges. Animal Feed Science and Technology, 53, 173–187.

728 Ylinen, V., Pylkkö, P., Tuomola, E., Peura, J., & Valaja, J. (2018). Performance in blue fox (Vulpes
729 lagopus) fed low-protein diets supplemented with DL-methionine and L-histidine. Agricultural and
730 Food Science, 27, 168–178. https://doi.org/10.23986/afsci.73149

731

732